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Ethanol and Some Tetrahydroisoquinolines Alter the Discharge of Cortical and Hippocampal Neurons: Relationship to Endogenous Opioids

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BERGER, T., E. D. FRENCH, G. R. SIGGINS, W. T. SHIER AND F. E. BLOOM. *Ethanol and some tetrahydroisoquinolines alter the discharge of cortical and hippocampal neurons: Relationship to endogenous opioids.* PHARMAC. BIOCHEM. BEHAV. 17(4)813–821, 1982.—The activity of single neurons in rat cortex or hippocampus (HPC) was recorded to test two hypotheses: (1) neuronal effects of ethanol are mediated by an endogenous opiate-like mechanism (for example, by release of an endogenous opioid peptide), and; (2) ethanol-induced formation of aldehyde-catecholamine condensation products (tetrahydroisoquinolines; TIQs) might contribute to some acute actions of ethanol. Ethanol and all TIQs were applied to single neurons from multibarrel micropipettes by electroosmosis or pressure. Ethanol most often inhibited neurons of the parietal cortex, while activating most HPC pyramidal neurons. Tetrahydropapaveroline (THP) most often inhibited the spontaneous and glutamate- or acetylcholine (ACh)-induced firing of neurons in both these regions, although some excitations were also seen. In contrast, salsolinol and 7-O-methyl-salsolinol predominantly excited HPC pyramidal neurons, but depressed most parietal cortical neurons. Iontophoretic or SC naloxone usually antagonized the excitatory actions of ethanol, salsolinol and methionine^5-enkephalin on HPC pyramidal cells; however, ACh-induced speeding also was antagonized occasionally. Conversely, the antimuscarinic agent scopolamine antagonized the excitatory actions of salsolinol, but not those of met-enkephalin, in some HPC pyramidal cells. These results and those of previous studies show that acutely applied ethanol or salsolinol elicits a region-specific pattern of neuronal effects in brain similar to that previously described for opiates: activity is inhibited in several tested brain areas but excited in hippocampus. Furthermore, these excitatory effects are antagonized by naloxone. However, because of the occasional apparent non-specific effects of naloxone and the puzzling antagonism of the salsolinol-induced excitations by scopolamine, some doubt remains whether the opiate-like actions of these substances can be completely attributed to mediation by opiate receptors.

Ethanol Salsolinol Tetrahydropapaveroline Naloxone Hippocampal Pyramidal neurons Opiates

IN spite of considerable research, little is known about the cellular mechanisms involved in either acute ethanol intoxication or dependence. Potential insight into human alcohol dependence was provided by the tetrahydroisoquinoline (TIQ) hypothesis [7,12], that later was supported by reports of TIQ-induced increases of voluntary ethanol consumption in selected rats [27–29]. Although it has not been resolved whether TIQs are formed *in vivo* in the brains of animals treated only with ethanol (see [19,51] vs. [35, 40, 52]), TIQs are formed *in vivo* and *in vitro* after ethanol treatment and administration of monoamine related drugs [6–8, 12, 28, 49, 51, 52]. Furthermore, TIQs are found in the urine and cerebrospinal fluid of untreated human alcoholics [9,46] and could therefore be viewed as potential mediators of some effects of ethanol in these subjects.

One of the active TIQs [27–29], tetrahydropapaveroline (THP), is formed by the condensation of dopaldehyde and dopamine. Because THP is a precursor in the biosynthetic pathway to morphine, early speculation centered on whether the actions of ethanol might involve the intermediation of an opiate-like mechanism [12]. This hypothesis has gained credence from recent reports showing similarities [3, 5, 20, 43] and cross-tolerance [3,22] between the actions of ethanol and opiates, and the effectiveness of opiate antagonists in reduc-

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ing some actions of ethanol or TIQs [1, 3, 5, 20, 21, 23–25, 48].

However, to our knowledge, only one study [43] has described the electrophysiological actions of these TIQs on central mammalian neurons; such actions might help to explain the cellular mechanism of the behavioral effects. There have been no systematic attempts to determine if the central electrophysiological actions of ethanol or TIQs can involve endogenous opioid peptides or opiate receptors. Sufficient reports on the electrophysiological actions of opioids on the neurons of many brain regions have appeared [33, 34, 54] to warrant comparison to ethanol and TIQ effects. Moreover, hippocampal pyramidal cells show a unique excitatory response to iontophoresis of the opioids [33, 45, 53] that we believe derives from a disinhibition resulting from depression of neighboring interneurons [32, 44, 45, 53]; this excitatory response could provide a key comparison for ethanol or TIQs effects. Furthermore, the effects of ethanol on the electrophysiology of the hippocampus have been the subject of many papers and preliminary reports [4, 10, 11, 13, 18, 30, 31, 36, 42, 47]. Therefore, in the present study we have tested the actions of locally-applied ethanol and three TIQs, salsolinol, 7-O-methylsalsolinol and THP, on single hippocampal and cortical neurons, to determine: (1) whether TIQs affect neuronal firing in these areas; (2) whether ethanol effects are replicated by any of the TIQs; (3) whether these substances mimic the naloxone-reversible actions of locally-applied opioids. These TIQs were chosen because of their behavioral effects [27–29] and their possible formation *in vivo* after alcohol administration [6–9, 12, 19, 49, 51]. Some of our results have been reported elsewhere in preliminary form [15, 41, 42].

METHOD

Male Sprague-Dawley rats (n=48) weighing 150–300 gm were used in all studies. Anesthesia was induced by 3% halothane in air; following tracheotomy and other surgery, light anesthesia was maintained (0.75–1% halothane-air mixture) by periodically adjusting the depth to allow slight spontaneous vibrissal movements. The animals were mounted in a stereotaxic frame and a small hole (3–4 mm dia.) was made in the cranium (P2.0–P3.0 mm and L2.0–L3.0 mm from bregma) for recording from parietal cortex and the underlying dorsal hippocampus (2.2–3.7 mm below dura). Electrode penetration of this area was facilitated by gently lifting up the dura with a small suture needle and cutting it with iridectomy scissors.

Single-unit extracellular recordings were made through the central barrel (containing 3 M NaCl, 2–5 MΩ) of a 5-barrel micropipette. Overall tip diameter of the micropipettes measured 4–7.5 μm. Solutions and pipettes were prepared immediately before the experiments as previously described [16, 33, 43]. The drugs used were as follows: L-glutamate (0.5 M, pH 8), acetylcholine (ACh) chloride (2 M, pH 4), tetrahydropapaveroline HCl (THP), salsolinol HCl (SAL), 7-O-methylsalsolinol HCl (7-MeSAL; all three TIQs were 0.003 M in 0.165 M NaCl, pH 5.5–6.5), scopolamine HBr (0.1 M, pH 5), naloxone hydrochloride (0.1 M, pH 4), Met^5-enkephalin (0.03 M in 0.165 M NaCl, pH 4) and ethanol (1–3 M in 0.165 M NaCl, pH 7). Met-enkephalin was synthesized and supplied by Dr. N. Ling (Salk Institute). SAL, 7-MeSAL and THP were synthesized by one of us (T.W.S.) at the Salk Institute. To minimize artifactual drug responses due to current or to pH or osmolarity effects, the TIQs, Met-enkephalin and ethanol were ejected either by pressure or by electroosmosis with positive current, and retained in the barrel by 3–5 nA of negative current. Generally, 2–3 M ethanol was used only when electroosmosis alone was planned (about 10% of experiments). All other drugs except glutamate were retained with 10–20 nA negative current and ejected with positive current. Glutamate was retained with 10–20 nA of positive current and ejected with negative current.

Three outer barrels of the pipette assembly were filled with drugs while the fourth barrel was filled with 3 M NaCl and used for current neutralization and control [14,16]. Drugs were ejected by means of a BH-1 iontophoresis circuit (Medical Systems, Inc.) with automatic current neutralization. A modification of a micro-pressure ejection system [26,39] was also devised to be used in conjunction with iontophoretic drug applications, using the same 5-barrel iontophoresis micropipettes: the drug-containing barrel was connected with a metal pressure tube that also served to pass current for iontophoresis as well as allowing drug retention by current to operate between periods of drug ejection. At times both pressure and current ejection were used concurrently. The micropressure method is especially advantageous for use with poorly ionized or relatively dilute compounds. In all cases in which micropressure was employed, an effort was made to maintain solutions at or near neutral pH; this did not effectively alter their current carrying ability. However, for pressure application, SAL and THP were maintained at pH 6–6.5, because these compounds tend to change color (indicating oxidative) at higher pH values.

Unitary action potentials were displayed on an oscilloscope and separated from background noise (bandpass, 1–10 kHz) by a voltage gating window discriminator, the square pulse output of which was integrated over 1 sec intervals and recorded as firing rate on a polygraph. The DC signal at the electrode tip was also recorded on the polygraph as an additional monitor of current imbalance. In addition to the ratemeter record, drug effects were also quantitatively analyzed by a PDP 11/40 computer (DEC), which generated peri-drug response time histograms (FISH.MAC software of K. Liebold), similar to post-stimulus time histograms but using regularly-timed drug ejection pulses instead of pathway stimulation [14,16].

A neuron was considered to be responsive to a drug if: (1) the firing rate changed by 20% or more with full recovery after termination of drug application, (2) the response could be repeated at least twice, (3) the net current imbalance was less than 10 nA, and (4) the response was not seen with ejection of identical currents through the NaCl (current balance) barrel. A response was considered partially antagonized if naloxone or scopolamine reduced it by 50% or more. In such drug interaction tests, agonists were always applied in pulses of constant current and duration, at regular uninterrupted intervals. Naloxone was administered either by iontophoresis or by subcutaneous injection of 2–10 mg/kg. When administered by iontophoresis, naloxone was first applied at low, sub-effective currents (no holding current to 5 nA) and the current gradually increased until agonist responses or background firing were altered. If background rate was altered, the test was discarded.

Initially, at the end of all hippocampal experiments, verifications of electrode placements were made by histological localization of either Fast Green (saturated, in 3 M NaCl) or Pontamine Sky Blue (2% in 0.5 M Na acetate) dye passed from the recording barrel. Because virtually all electrode

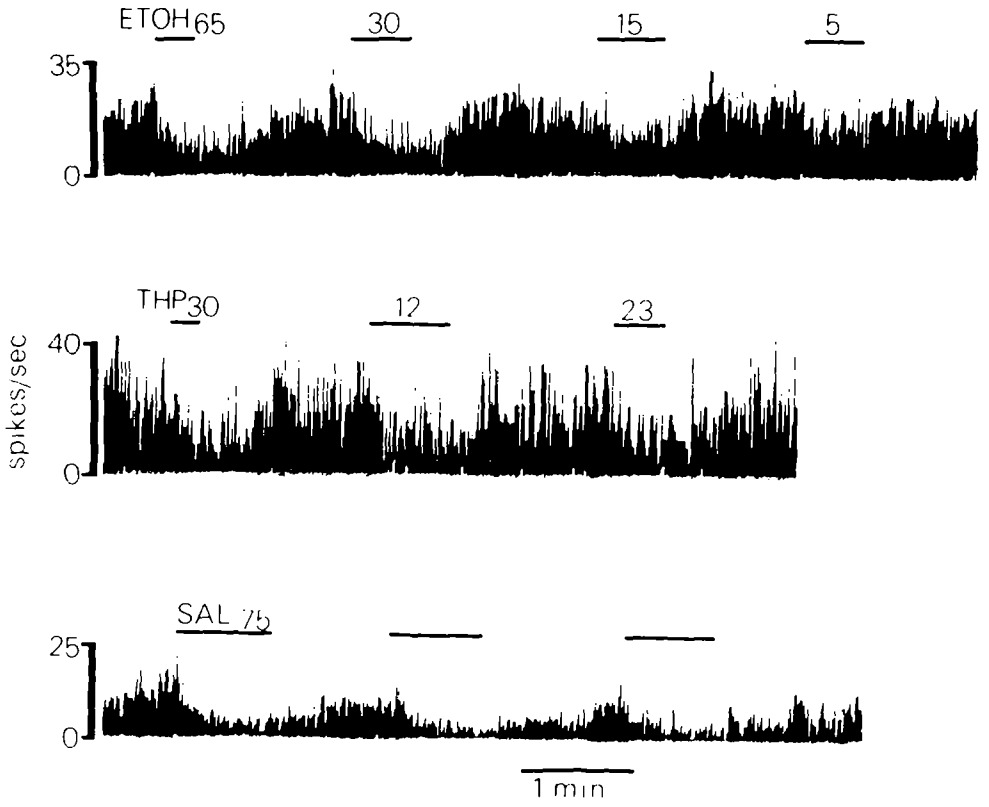

FIG. 1. Inhibitory effects of ethanol, THP and salsolinol on unidentified cortical neurons. Each panel represents ratemeter polygraph records, taken from three different cells. In this and all subsequent figures, bars over the records indicate the duration of drug application. In this figure all drugs were ejected from five-barrel micropipettes by means of electroosmosis. The numbers above each bar refer to nA of ejection current. Note dose-response relations for ethanol and THP. In the lower panel, SAL was applied only at one dose, 75 nA. Ethanol was 1 M in the pipette.

placements were within the hippocampus in these experiments, and because animals of similar size were used, subsequent hippocampal experiments utilized neuronal firing patterns, electrode depth, stereotaxic coordinates, and occasional histological checks for placement verification.

RESULTS

In the tests of agonists on cortical and hippocampal neurons, an attempt was made to minimize artifactual responses due, for example, to current, pH or osmolarity effects by the controls noted in the methods section, and by applying the agonists in two unrelated ways—microelectroosmosis and pressure. Cases where one method revealed responses at variance with the other, or where spike size was affected, were eliminated from data analysis. Most neurons were tested with at least two of the three agonists; testing of all three agonists on the same neuron was not always possible because of the occasional presence of antagonists in the third pipette barrel. The direction of agonist responses described below for either cortex or hippocampus did not appear to depend on the firing rate of the cell under study.

Cortex Neurons

Forty nine unidentified neurons were studied in the parietal cortex overlying the dorsal hippocampus. Figure 1 shows representative inhibitory actions of ethanol, THP and SAL on three spontaneously firing cortical cells. Table 1 indicates that the spontaneous firing of the majority of responsive cells in the cortex was inhibited by ethanol, THP and SAL, regardless whether these substances were applied by micro-electroosmosis or by pressure. At concentrations of 1 M in the pipette barrel, ethanol produced somewhat weaker responses by electroosmosis; 2–3 M gave more

TABLE 1

EFFECTS OF LOCALLY-APPLIED ALCOHOL AND TIQs ON SPONTANEOUS NEURONAL ACTIVITY IN PARIETAL CORTEX (UNIDENTIFIED CELLS)

Substance	N Cells	Speeding*	Slowing*	No Change*
EtOH 1-3 M	11	45	55	0
SAL 3 mM	22	27	55	18
THP 3 mM	16	0	62	38

*Percent total cells.

TABLE 2

EFFECTS OF LOCALLY-APPLIED ALCOHOL AND TIQs ON SPONTANEOUS NEURONAL ACTIVITY IN HIPPOCAMPUS

Substance	N Cells	Speeding*	Slowing*	Biphasic or Reversible*	No Change*
EtOH 1–3 M	31	55	32	10	3
SAL 3 mM	150	73	15	3	9
7-Me SAL 3 mM	9	56	33	0	11
THP 3 mM	20	40	45	10	5

*Percent total cells.

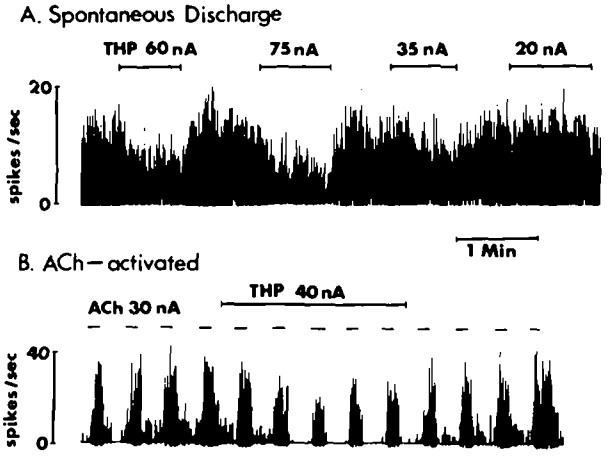

FIG. 2. Inhibitory effect of THP, applied by electroosmosis, on two different hippocampal pyramidal neurons. A: THP inhibits the spontaneous discharge of this neuron in a dose-dependent manner. B: THP inhibits the acetylcholine (ACh)-induced activity, as well as the slow background firing, of another, nearly electrically quiescent neuron. ACh was applied in short, regularly-repeated pulses of 30 nA.

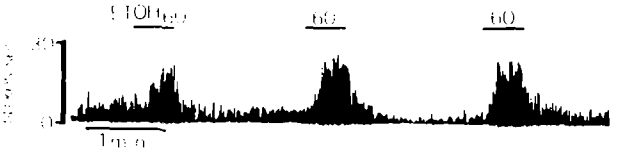

FIG. 3. Ethanol (ETOH) excites a hippocampal pyramidal neuron. Ethanol was applied by electroosmosis, 60 nA. This neuron showed slow, somewhat variable spontaneous background activity that accounts for changes in interpulse firing frequency. Ethanol was 1 M in the pipette.

robust responses. In contrast, the depressions produced by THP and SAL were pronounced in spite of maximum concentrations in the pipette of only 3 mM.

Hippocampal Neurons

More than 200 neurons were studied in the dorsal hippocampus. Most of these were identified tentatively as pyramidal cells on the basis of their burst-like spontaneous firing patterns, their depth from the cortical surface, their stereotaxic coordinates and subsequent histological identification. Most of these neurons were recorded in CA3 and the lateral part of CA1.

As in cortex, THP in the hippocampus was most likely to depress spontaneous (Table 2) or acetylcholine-induced firing (Fig. 2), although excitation was also seen in 40% of cells. Ethanol and 7-MeSAL also had mixed effects, although here enhancement of firing rate predominated over depression of firing (Fig. 3); in addition, biphasic responses to ethanol, usually with early excitation giving way to later depression, were seen in 10% of cells. SAL showed more consistent excitation of hippocampal neurons (73%; Table 2). Effective doses were generally comparable to those in cortex. Excitatory responses to ethanol or to the salsolinols were not accompanied by a change in spike size.

Naloxone

Because we found hippocampal neurons to be predominantly excited also by locally applied opiates and opioid peptides [33, 45, 53], we also tested the influence of naloxone against the excitatory actions of ethanol, SAL and acetylcholine. Acetylcholine was applied in alternate pulses with either ethanol or SAL to serve as a control for specificity. Naloxone antagonized most of the excitations to either ethanol (a total of 75% of 8 cells studied) or SAL (89% of 37 cells; Table 3 and Figs. 4 and 5). Such antagonism was seen without a significant change in basal firing rate and regardless of the route of naloxone administration. Subcutaneous naloxone (2–10 mg/kg) antagonized 2 of 3 ethanol, and 3 of 4 SAL responses. In 10 cells, naloxone antagonism of SAL was found to parallel antagonism of Met^5-enkephalin (Figs. 4 and 5). However, in 6 of 18 cells showing complete block of SAL effects, the excitatory response to acetylcholine was also measurably antagonized by naloxone applied by iontophoresis (Table 3). Nonetheless, the remaining 12 cells showed a clear naloxone antagonism of SAL effects without influence on acetylcholine responses.

Scopolamine and Mg^{++}

Because SAL produced predominantly naloxone-sensitive excitations in the hippocampus much like those produced by the opioid peptides [33, 45, 53], this TIQ was

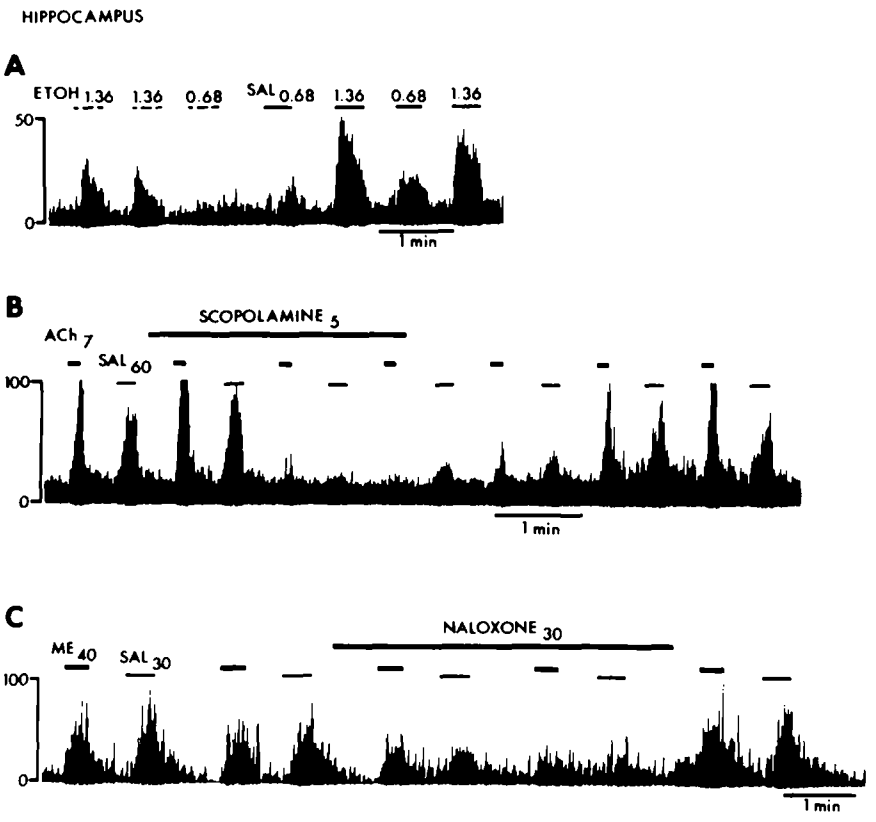

FIG. 4. Responses of hippocampal neurons to ethanol and SAL and their interactions with drug antagonists. A: Excitatory effect of ethanol and SAL applied by pressure; numbers above bars indicate pressure in atmospheres. Note rough dose-response relationships. Ethanol was 1 M in the pipette. B: The anti-muscarinic agent scopolamine (applied by iontophoresis; 5 nA) simultaneously blocks the excitatory action of both acetylcholine (ACh) and SAL. Also note the nearly simultaneous recovery of responses to both agonists; this amount of scopolamine has little direct effect on basal firing rate. C: Naloxone (applied by iontophoresis: 30 nA) simultaneously blocks the excitatory actions of both methionine^5-enkephalin (ME) and SAL. Both agonists were applied by electroosmosis. Note the simultaneous recovery of the agonist responses after termination of the naloxone current, and the lack of direct inhibitory effect (or even, perhaps, a slight excitatory effect) of this dose of naloxone on basal firing rate.

TABLE 3

EFFECTS OF NALOXONE ON NEURONAL RESPONSES TO LOCALLY-APPLIED ALCOHOL AND SAL IN HIPPOCAMPUS

		Percent of Cells; Agonist Responses: Antagonized		
Agonist	N Cells	Partial	Complete	Not Affected
EtOH 1–3 M	8	25	50	25
SAL 3 mM	37	51*†	38‡	11

*In 6 of these cells iontophoretically applied naloxone also 'non specifically' antagonized ACH responses.

†In 5 of these cells naloxone blocked both SAL and Met-enkephalin.

‡In 5 of these cells naloxone antagonized both SAL and Met-enkephalin.

studied more extensively. It has been shown [16] that the anti-muscarinic agent scopolamine selectively blocks excitations evoked by acetylcholine in the hippocampus, without affecting those produced by the opioids. However, in the present study, the excitatory effects of SAL, but not those of glutamate, also were clearly antagonized (Fig. 6) in 73% of presumed pyramidal cells (n=11) by the concurrent iontophoretic application of scopolamine (1 to 10 nA).

Iontophoresis of magnesium ions was also tested against the SAL responses, because it has been shown that Mg^{++} can block the excitatory effect of the opioids on hippocampal pyramidal neurons [45,53], probably by blocking synaptic release of inhibitory transmitter(s) and thereby preventing a similar action of the opiates. However, in 11 of 11 neurons, iontophoresis of Mg^{++} ions (1 to 10 nA) had no selective effect against the excitatory responses produced by salsolinol (Fig. 7). At these doses, Mg^{++} always greatly elevated background firing rates, perhaps indicating antagonism

of the tonic release of inhibitory transmitters. Of some interest was the finding that Mg^{++} also occasionally antagonized the excitatory actions of acetylcholine.

DISCUSSION

As far as we are aware, this is the first report showing a neuroactive effect of TIQs on single cortical and hippocampal neurons. These actions could serve as the cellular substrates for the behavioral effects seen after central administration of these substances in awake animals. Our goal in this study was to test two hypotheses related to acute ethanol actions in the nervous sytem: (1) ethanol-induced formation of aldehyde-catecholamine condensation products (TIQs) might contribute to some acute actions of ethanol, and (2) some neuronal effects of ethanol are mediated by an opiate-like mechanism. The hippocampal pyramidal cell was chosen as an appropriate test site because it demonstrates a unique response to opioids and because catecholamines (potential substrates for TIQ formation) are present in hippocampus.

With regard to the first hypothesis, our data do not rule out the possibility that salsolinol (or some close analogue such as 7-MeSAL) could mediate the action of ethanol, by virtue of their comparable excitatory effects when applied directly to pyramidal cells. Moreover, comparison of all the TIQ data derived from this study, and those on cerebellum and caudate reported previously [43] indicates a fair mimicry between ethanol, SAL and opioid effects in terms of the predominant response pattern across four different brain regions [41]. Whereas some hippocampal neurons in the current study were excited by THP, the smaller percentage of THP excitatory responses compared to ethanol, SAL or 7-MeSAL actions suggests that THP is a lesser candidate for mediation of ethanol effects. Our finding that pipette concentrations of ethanol up to 1000-fold higher than those for SAL or 7-MeSAL were required to produce equivalent response magnitudes, could also be construed as support for a TIQ-mediation of ethanol effects. However, this suggestion must remain tentative, as intracellular recording will be required to determine whether the mechanism of action of ethanol and salsolinol is identical.

To support the second hypothesis requires the demonstration of a similar mimicry between ethanol and opiate actions, as well as a comparable pharmacological profile with respect to interactions between ethanol, opiates and specific antagonists such as naloxone. In terms of their effects on spontaneous extracellular unit activity in the hippocampus, locally applied ethanol and SAL show significant resemblances in comparison to the actions of opioids. One important similarity is the spectrum of actions of these substances across several brain regions. Thus, ethanol, THP and SAL, like the opioids, [33, 34, 54] appear to be predominantly inhibitory in most brain areas tested (except hippocampus), including cortex, caudate and cerebellum [41,43]. The opioids excite about 80% of hippocampal pyramidal neurons [33,53]. However, only SAL seems capable of exciting as large a proportion of hippocampal neurons as do the opioids. Whereas ethanol does activate a majority of hippocampal neurons, THP seems more inhibitory than SAL or ethanol.

Our results with locally-applied ethanol in the hippocampus differ with reports of a predominantly inhibitory action of systemic ethanol [18]. However, it should be noted that one study has revealed an enhancement by ethanol of both excitatory and inhibitory evoked activity [31]. Whereas preliminary extracellular field potential studies of hippocampal slices *in vitro* [11,13] indicate thanol-evoked depression of population spikes (with little effect on population synaptic potentials), extracellular and intracellular studies of the hip-

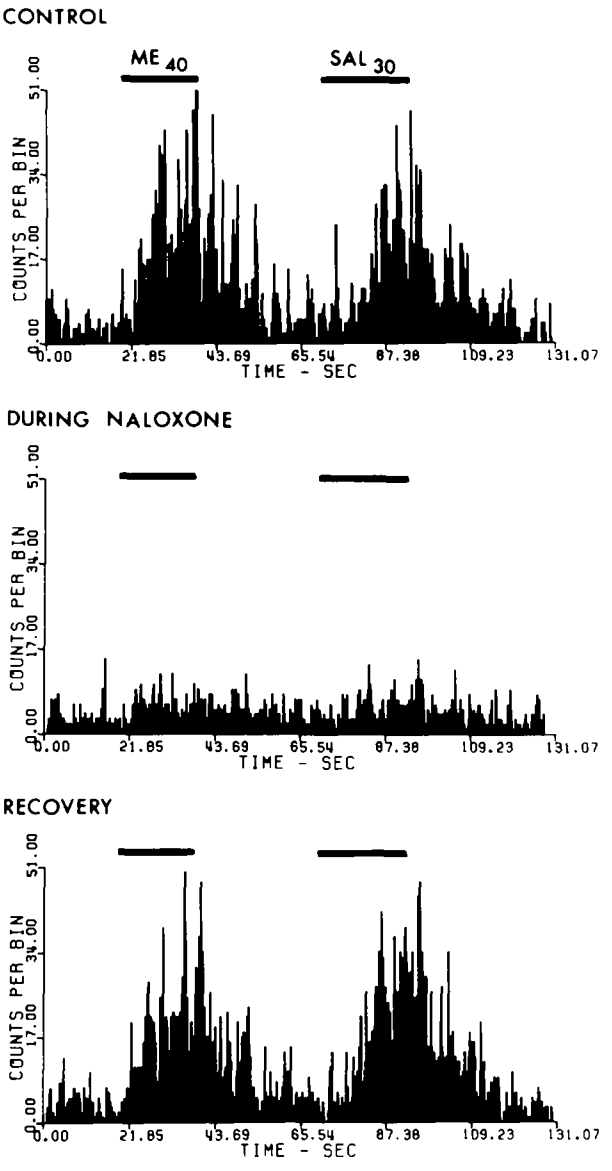

FIG. 5. Peri-drug interval histograms of unit excitatory responses to met^5-enkephalin and SAL and their blockade by naloxone in the hippocampus. Each histogram derived from 2 successive pairs of ME and SAL ejection pulses (electroosmosis) precisely time-locked to the sync-pulse initiating the computer analysis periods [14,16]. Histogram analysis programs (FISH.MAC;K. Liebold) indicate that firing rate is increased by over 500% of control by both ME and SAL in the control situation: these rate increases are highly significant at much greater than ± 1 S.D. However, during continuous iontophoresis of naloxone (30 nA; middle panel) the excitatory responses to both ME and SAL are reduced to the 2 S.D. level. The lower panel shows the complete recovery of the excitatory ME and SAL responses at one minute after termination of the naloxone current.

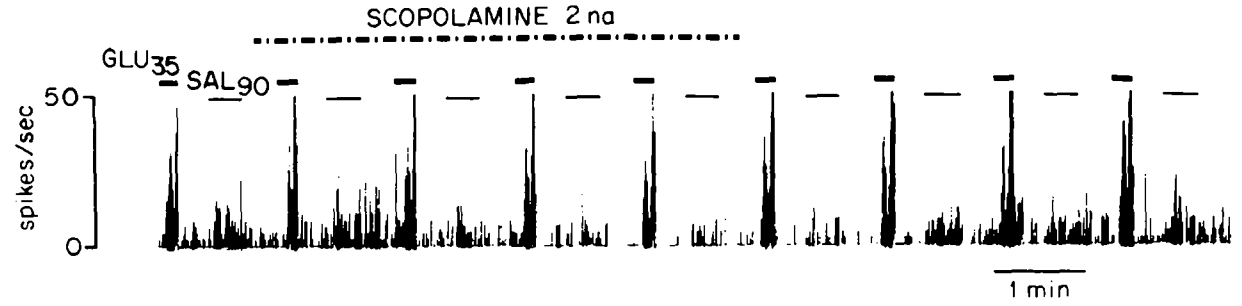

FIG. 6. Iontophoretically-applied scopolamine antagonizes the excitatory response of a CA3 hippocampal neuron to SAL (applied by electroosmosis) but not the response to iontophoretically-applied glutamate (GLU). However, note that this antagonism is not complete, and is accompanied by an apparent reduction in the basal firing rate.

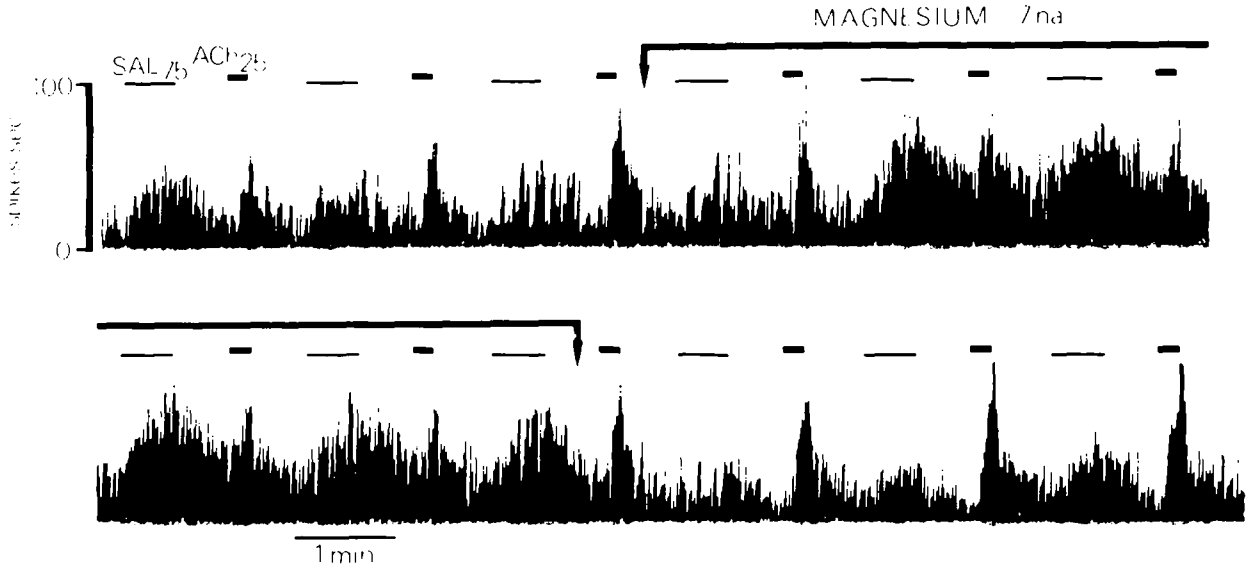

FIG. 7. Lack of effect of iontophoretically-applied magnesium on excitatory responses of a hippocampal neuron to SAL and ACh. Note that this dose of Mg^{++} directly elevates basal firing rate (as seen between the pulses of SAL and ACh).

pocampal slice in our laboratory often show an early excitatory action of ethanol ([36] Siggins, Pittman and French, in preparation). These excitations often give way within minutes to clear depressions of activity. Similar early excitatory, late inhibitory actions of ethanol have also been seen (S. Sorensen and B. Hoffer, personal communication) with ethanol applied by micropipette to hippocampal neurons of the mouse *in vivo*.

Another important similarity between the effects of ethanol, SAL and the opioids in the hippocampus is their sensitivity to antagonism by the opiate antagonist naloxone. Thus, similar percentages of the excitatory responses to ethanol, SAL and the opioids [33,48] are antagonized by either parenteral or iontophoretically-applied naloxone. However, it will be noted that rather high, perhaps unspecific, parenteral doses of naloxone (2–10 mg/kg) were required to antagonize the ethanol or SAL responses. These high doses may indicate that ethanol and SAL effects are mediated by actions not solely attributable to a pure opiate receptor. However, it is also possible that ethanol and SAL act at some opiate receptor subpopulation, such as the delta or kappa receptors, that require higher naloxone concentrations for antagonism. The fact that similarly large parenteral doses of naloxone (2–8 mg/kg) are required to antagonize iontophoretic enkephalin and endorphin responses [33] lends credence to this idea. Alternatively, large parenteral naloxone doses generally may be required to antagonize the effects of iontophoretically-applied opiates: the large opiate concentrations likely to arise at receptors nearest the pipette tip could require abnormal levels of naloxone for competitive antagonism.

However, in spite of these qualitatively similar actions and naloxone antagonisms, the opiate-mediation hypothesis is muddied by results of other experiments. First, in the present study efforts to block excitatory ethanol and SAL effects selectively with naloxone were occasionally confounded by an apparent antagonism (in 6 of 18 cells) of excitatory ACh effects by iontophoretically applied naloxone. However, this antagonism may not be non-specific, because we found earlier that naloxone, in iontophoretic amounts

sufficient to block opioid effects completely, did not antagonize the excitatory actions of glutamate [16, 33, 53]. Nevertheless, there is no known pharmacological basis for such naloxone antagonism of cholinergic receptors, although the recent description [2, 37, 50] of a disinhibitory mechanism of muscarinic effects in hippocampus, much like that observed for opiate actions [44, 45, 53], is suggestive: the presence of inhibitory opiate [53] and cholinergic [38] receptors on the same population of tonically firing inhibitory interneurons could lead to the observed results, if endogenous opioids were tonically released. Then, naloxone blockade of opioid receptors could effectively lead to reduced inhibition of the interneuron by exogenous ACh.

Further differences were seen with respect to the effects of salsolinol and opioids in the hippocampus. Thus, whereas the anti-muscarinic agent scopolamine has little action on opioid effects [16], SAL-induced excitations were frequently blocked by this agent. The meaning of this finding in terms of the mechanisms of action of either ACh or SAL is presently unclear, although some interrelationship between SAL and receptors to ACh may be indicated (see above).

A final difference between SAL and opioid effects was shown by studies with iontophoretically applied Mg^{++}. Whereas opioid excitations in the hippocampus were blocked by Mg^{++} [45,53], the excitations to salsolinol were not. However, the possibility cannot be excluded that synaptic transmission was not completely blocked in the present study, even though the increased firing rate during Mg^{++} application might indicate an efficacious antagonism of endogenous inhibitory transmitter release.

In conclusion, while the results of some of our experiments could be taken to support the hypothesis that some acute actions of SAL, and to a lesser extent ethanol, may involve opiate receptors (or perhaps release of opioid peptides) in the hippocampus, other findings confound this interpretation. Intracellular experiments are now under way to determine the degree of mimicry between the mechanisms of action of opioids, ethanol and SAL.

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